Synthesis and Antitumor Activity of 20(*S*)-Camptothecin Derivatives: Carbamate-Linked, Water-Soluble Derivatives of 7-Ethyl-10-hydroxycamptothecin[1)]

Seigo SAWADA,*[,a] Satoru OKAJIMA,[a] Ritsuo AIYAMA,[a] Ken-ichiro NOKATA,[a] Tomio FURUTA,[a] Teruo YOKOKURA,[a] Eiichi SUGINO,[b] Kentaro YAMAGUCHI[c] and Tadashi MIYASAKA[c]

Yakult Institute for Microbiological Research,[a] Yaho 1796, Kunitachi-shi, Tokyo 186, Japan. Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University,[b] Fukuyama-shi, Fukuyama, Japan and School of Pharmaceutical Sciences, Showa University,[c] Hatanodai, 1-5-8, Shinagawa-ku, Tokyo 142, Japan. *Received December 20, 1990*

Novel 36 derivatives (6), bonding the phenolic hydroxyl group of 7-ethyl-10-hydroxycamptothecin (4) with diamines through a monocarbamate linkage, were synthesized and their antitumor activity was evaluated *in vivo*. The derivatives were soluble in water as their HCl salts with the E lactone ring intact and exhibited significant antitumor activity. One of the derivatives, 6-27 showed excellent activity against L1210 leukemia and other murine tumors. The structure of its hydrochloride trihydrate (CPT-11) was determined by spectroscopic and crystallographic methods.

Keywords 20(*S*)-camptothecin; 7-ethyl-10-hydroxycamptothecin; carbamate; CPT-11; X-ray crystallography; antitumor activity

20(*S*)-Camptothecin (**1**, Chart 1) is an alkaloid which was first isolated from *Camptotheca acuminata* (Nyssaceae) by Wall and his co-workers in 1966.[2)] It has attracted much attention because of its significant inhibitory activity towards L1210 leukemia in mice and Walker 256 sarcoma in rats. The clinical trials of this alkaloid were carried out using its water soluble sodium salt, in which the E-lactone ring was cleaved by sodium hydroxide, but its severe toxicity to bone marrow and bladder ruled out the salt for the cancer treatment.[3)]

Camptothecin (lactone) administered in suspension exhibited a satisfactory high cytotoxic activity towards L1210 leukemia *in vivo* whereas the salt in aqueous solution has rather low activity. The lactone moiety of the alkaloid including the 20-hydroxyl group was said to be the responsible group for the activity,[3)] and the salt was assumed to lactonize *in vivo* although detailed behavior of the salt was not yet clear.

We describe here the synthesis of derivatives of 7-ethyl-10-hydroxycamptothecin (**4**), whose 10-hydroxyl group is connected with a hydrophilic functional side chain through a carbamate linkage, and the evaluation of their antitumor activity. We already reported the significant antitumor activity of the compound obtained herein, **6-27** (its hydrochloride trihydrate; CPT-11), toward various tomor systems[4)] and CPT-11 is now under phase II of clinical trials in Japan.[5)]

Chemistry

We reported the synthesis and antitumor activity of various 7-*C*-substituted and the A-ring modified derivatives starting from 20(*S*)-camptothecin.[6)] Compound **4**, which has a phenolic hydroxyl group as a foothold for hydrophilic modifications, was selected as the starting material because of its good therapeutic index [TI: optimal dose/minimum effective dose, **4**: TI=50, **1**: TI=3.1, L1210 in mice]. Compound **4** is also a potent inhibitor of deoxyribonucleic acid (DNA) topoisomerase I.[7c)]

Synthesis of **4** was depicted in Chart 1. Hydrogen peroxide was added dropwise to an ice-cold solution of **1** in sulfuric acid in the presence of ferrous sulfate and freshly distilled propionaldehyde. From the reaction mixture **2** was obtained in a good yield. Compound **2** was converted into its *N*-oxide (**3**). The *N*-oxide was then dissolved in dioxane containing equimolar amounts of sulfuric acid, and the mixture was irradiated with a high pressure Hg lamp (450 W) through a Pyrex filter to afford the 10-hydroxyl compound (**4**) in 30—35% overall yields.[1)]

Since the introduction of a bulky substituent either in the A or in the B-ring of camptothecin weakened or completely vanished its activity, a hydrophilic side chain is bound to the phenolic hydroxyl group of **4** through such linkages as organic or inorganic esters, glycosides, which seemed to be susceptible to enzymatical hydrolysis *in vivo*. We at first attempted to synthesize the derivatives having an amino acid group as the side chain, but these carboxyester derivatives decomposed to the starting phenol during purification. We selected more stable carbamate linkage, which was hydrolyzable *in vivo*.[8)] Furthermore, the fact[9)] that the linkage is selectively hydrolyzed in some tumor cells attracted our interest for a homing device.

A series of carbamate derivatives (**6**) were prepared either by the reaction of chlorocarbonyl derivatives of diamines with **4** in pyridine, or by the reaction of diamines with chlorocarbonyloxy compound **5**, which was obtained in a good yield by the treatment of **4** with phosgen in the presence of triethylamine (Chart 1 and Table I). In this case, the phenol **4** was yielded in addition to **6**. The secondary–secondary or secondary–tertiary diamines used in this work were mostly commercially available, but acyclic diamines and 4-dialkylaminopiperidines were prepared according to the reported methods.[10)] The yields were fairly low in the reaction of **4** with isocyanates and the reactions of **5** with primary–secondary or tertiary diamines and amino acid esters, since the products seemed to be hydrolyzed *via* a deprotonation–elimination mechanism in the basic reaction media.

CPT-11, hydrochloride trihydrate of **6-27**, was chosen for the determination of its structure. CPT-11 was obtained as slightly pale yellow needles or crystalline powder by crystallization from water and the compound has three molecules of water of crystallization suggested by its elemental analysis. The crystals were stable to heat (100 °C for a week) but some degradation was observed by exposure to visible light. The salt dissolved in water into a clear acidic solution (pH 4 in 2% solution) in which the compound was

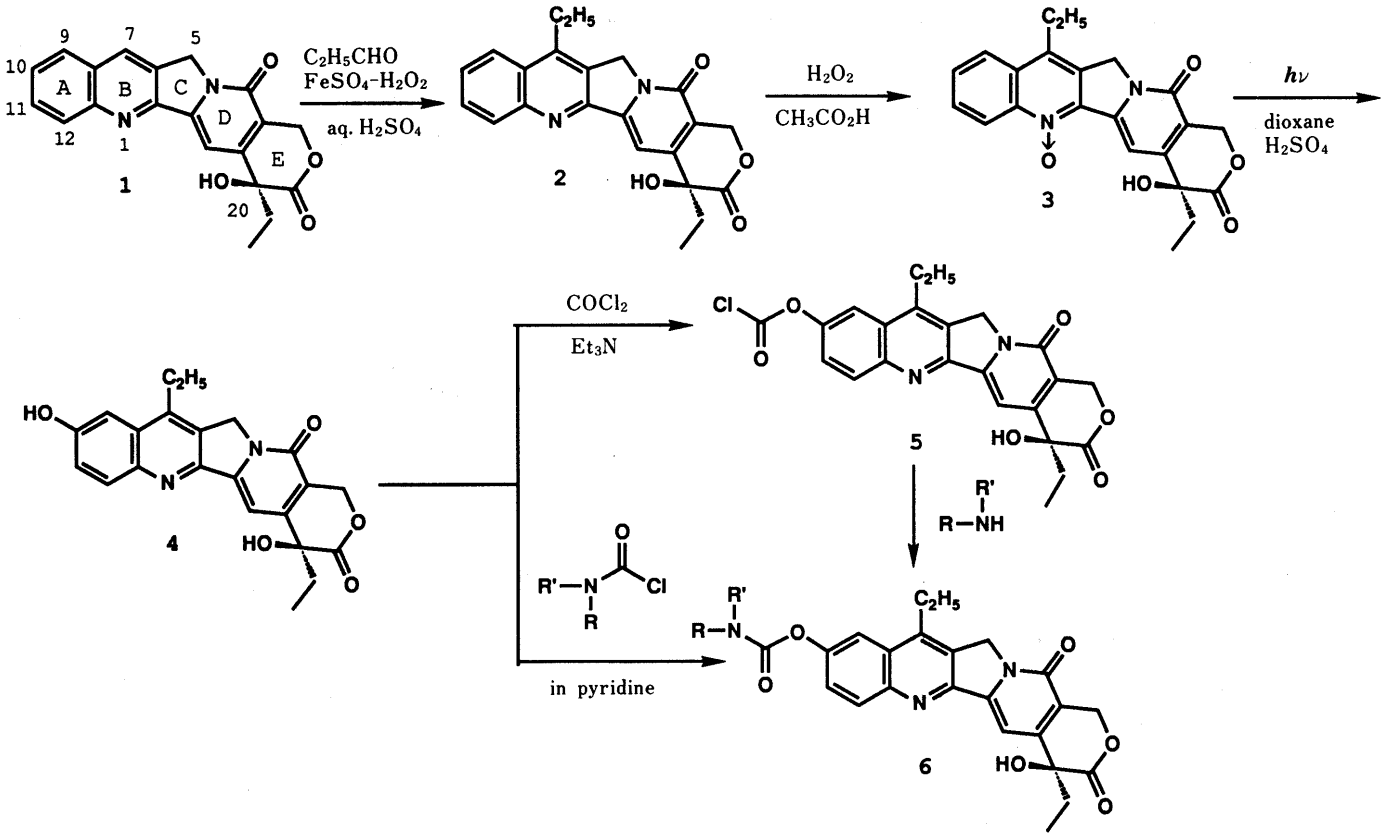


Chart 1

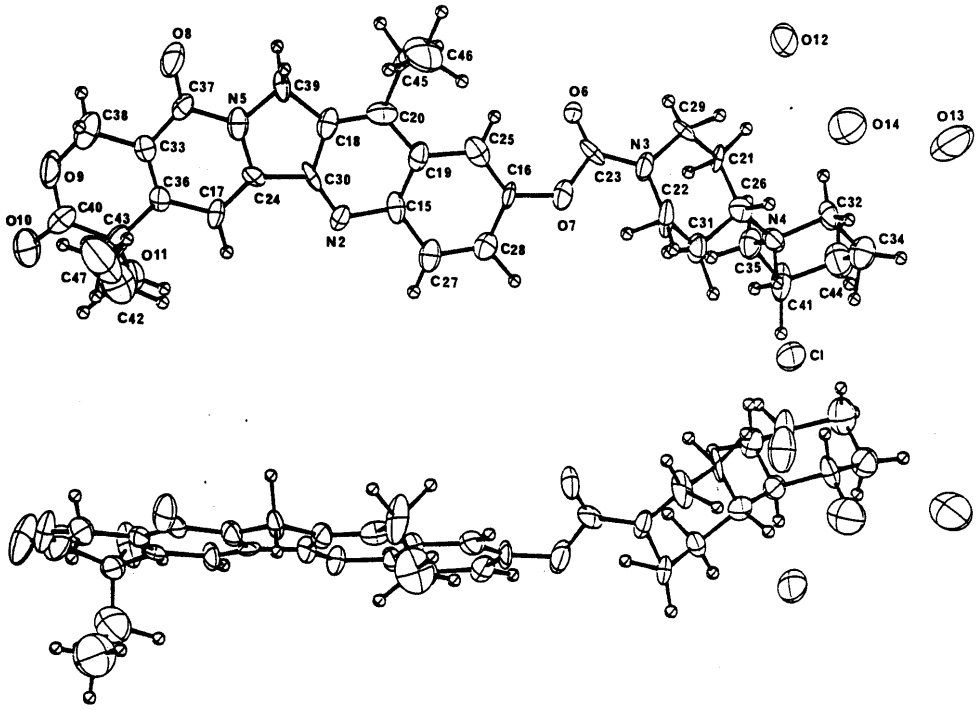


Fig. 1. ORTEP Drawings of CPT-11 from a Different View

stable (100 °C for 16 h) under the interception of light. The full assignments of proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy by homo and hetero correlation spectroscopy (COSY) and correlation spectroscopy *via* long range coupling (COLOC) methods, and also other spectroscopic data, were met in Experimental.

The proposed structure was confirmed by the single crystal X-ray crystallographic study. The observed cell parameters for a crystal of CPT-11 ($0.35 \times 0.05 \times 0.20$ mm) recrystallized from aqueous acetonitrile were as follows: $C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$, $M_w = 677.19$, orthorhombic,

TABLE I. Antitumor Activity of the Camptothecin Derivatives on L1210 Leukemia in CDF_1 Mice

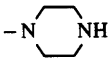
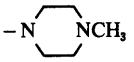
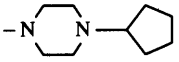
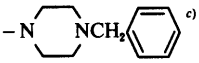
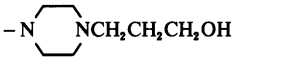
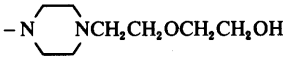
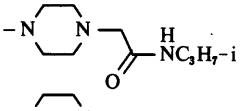
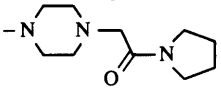
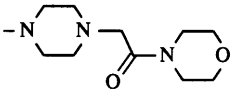
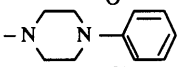
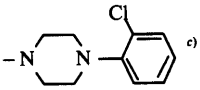
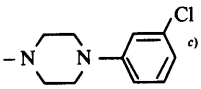
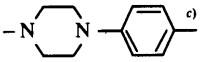
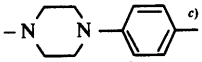
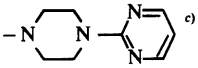
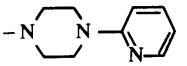
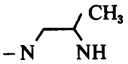
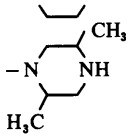
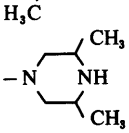
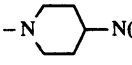
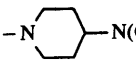
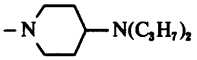
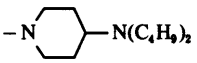
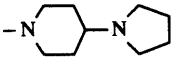
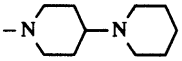
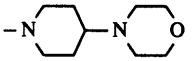
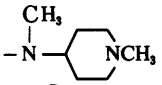
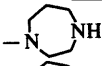
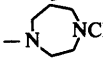
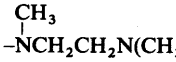
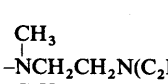
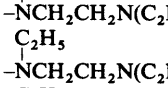
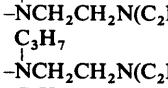
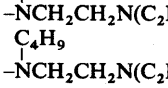
Compd. 6 Diamine side chain	Survival time of control animals (d, mean±S.D.)	T/C%[a] Total dose (mg/kg) 1.56	3.12	6.25	12.5	25	50	100	200	400
01 	7.0±0.0	—[b]	111	124	131	167	210	226	352	552 (5/6)[d]
02 	7.0±0.0	114	121	119	129	133	176	217	—	—
03 	7.0±0.0	114	114	121	121	138	188	238	293	—
04 	7.0±0.0	119	121	124	126	124	136	186	205	—
05 	7.2±0.4	—	—	—	—	127	134	167	211	—
06 	6.7±0.5	128	137	172	201	287	520 (4/6)	122	60	—
07 	7.0±0.0	—	114	119	119	127	137	164	197	269
08 	7.0±0.0	—	—	—	—	126	126	138	143	—
09 	7.0±0.0	—	112	119	119	148	148	279	571 (6/6)	129
10 	7.3±0.5	116	119	123	123	129	139	167	434 (3/6)	162
11 	7.0±0.0	114	114	124	126	124	129	131	148	229
12 	6.3±0.5	119	122	135	148	153	161	169	183	—
13 	7.2±0.4	—	—	—	—	380 (3/6)	556 (6/6)	286 (2/6)	88	—
14 	7.2±0.4	—	—	—	—	130	150	183	245	—
15 	7.0±0.0	—	—	—	—	213	299 (1/6)	500 (5/6)	104	—
16 	7.0±0.0	—	—	—	—	114	129	152	193	—
17 	7.5±0.5	—	—	—	—	307 (2/6)	387 (3/6)	347 (3/6)	107	—
18 	7.2±0.4	—	—	—	—	169	167	206	396 (2/6)	—
19 	7.0±0.0	—	—	—	—	140	167	198	302	—
20 	7.0±0.0	—	—	—	—	114	212	140	171	—
21 	7.0±0.0	—	—	—	—	124	133	143	193	—
22 	7.3±0.5	112	114	119	137	180	192	237	297	352 (1/6)
23 	7.0±0.0	—	—	—	—	143	205	250	302	—

TABLE I. (continued)

Compd. 6 Diamine side chain	Survival time of control animals (d, mean±S.D.)	$T/C\%$[a] Total dose (mg/kg) 1.56	3.12	6.25	12.5	25	50	100	200	400
24 	7.0±0.0	—	—	—	—	150	205	245	312 (1/6)	—
25 	7.0±0.0	—	—	—	—	154	207	229	340 (1/6)	—
26 	7.3±0.5	112	121	148	153	199	224	274	463 (4/6)	495 (4/6)
27 	7.0±0.0	—	119	129	167	226	274	571 (6/6)	571 (6/6)	124
28 	8.0±0.0	104	113	119	117	125	160	192	338 (2/6)	81
29 	6.3±0.5	100	98	127	130	130	132	148	178	—
30 	7.0±0.0	—	—	—	—	117	114	119	126	—
31 	7.0±0.0	—	—	—	—	129	155	198	298 (1/6)	—
32 	7.0±0.0	124	129	124	133	145	226	310 (1/6)	495 (5/6)	90
33 	7.0±0.0	—	—	—	—	114	157	183	236	—
34 	7.3±0.5	—	—	110	110	121	142	176	201	—
35 	7.5±0.5	109	118	118	116	113	127	153	180	—
36 	7.5±0.5	—	—	102	104	104	116	127	144	—

a) $T/C\%$ = (mean survival time of the tested animals)/(mean survival time of the control animals) × 100. b) Not tested. c) Administered in suspension. d) Number of cured mice/number of mice tested.

$P2_12_12_1$, $a=12.154$ (5), $b=42.847$ (5), $c=6.422$ (5) (Å), $V=3344(3)$ Å^3, $Z=4$, $D_x=1.345$ Mgm^{-3}, $\lambda(CuK\alpha_1)=1.54050$ Å, $\mu=1.521$ mm^{-1}, $F(000)=1440$, $T=295$ K. The structures were solved by the direct method, and refined by full-matrix least-squares calculations assuming anisotropic temperature factors for nonhydrogen atoms and isotropic ones for hydrogen atoms. Final $R=0.083$, $R_w=0.080$ for 1724 reflections above $3\sigma(F)$. Atomic coordinates and isotopic thermal parameters are shown in Table IV.

As illustrated in Fig. 1, the structure of the compound consists of a 7-ethylcamptothecin skeleton with a piperidinopiperidine side chain and the presence of a chlorine atom and three oxygen atoms demonstrated the hydrochloride trihydrate structure. The side chain has an intertwisted structure and the E ring lactone moiety constitutes a *quasi* chair form and the ethyl group at C_{20} installs in *quasi* axial and the free hydroxyl group is equatorial, of which conformation was similar to that of 20-*O*-iodoacetylcamptothecin.[11]

The importance of the 20(*S*)-geometry of camptotheca alkaloids for antitumor activity has been demonstrated in comparison with its antipode in the animal tumor system.[12] We examined the optical purity of compound **6-27** by NMR spectrum using a shift reagent, {tris-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate]-europium (III)}. The hydrogen at the 9-position of compound **6-27** synthesized starting from 20(*S*)-camptothecin, has one doublet at δ 7.78, whereas the racemic **6-27**, synthesized according to Wall's report,[13] showed two doublets at δ 7.78 and δ 7.80 ppm arising from *S* and *R* form, respectively. A chiral stationary phase high performance liquid chromatography (HPLC) separation of CPT-11 also indicates that there was no detectable *R*-form.

Results and Discussion

The activity of novel 36 derivatives synthesized in this work was estimated toward L1210 leukemia in mice (Table I). The test was carried out using a clear aqueous solution of their HCl salt, but the compounds **6-06**, **13**—**17**, and **18** were administered in suspension because of the low solubility of the HCl salts of these compounds. The HCl salt of **6-23** gave compound **4** as a precipitate in the aqueous solution. All the water soluble derivatives exhibited the activity [$T/C\% > 125$ (the percentage of the mean survival time of the treated and that of the untreated group)]. Cured mice were observed in 9 derivatives, **6-01**, **09**, **10**, **22**, **24**—**27**, and **31** at a total dose of 100 to 400 mg/kg.

Table II. Antitumor Activity of the Selected Derivatives against Acites Tumors

Tumor system	Total dose (mg/kg)	T/C%[a]		
		6-01	6-09	6-27
P338 leukemia	3.12	141	111	144
i.p.–i.p.	6.25	156	115	165
q4d d 1—9	12.5	173	126	194
	25.0	187	146	235
	50.0	211	246	281
	100.0	265	393 (4/6)[b]	343 (1/6)
	200.0	404 (4/6)	404 (4/6)	428 (3/6)
	400.0	444 (6/6)	74	333 (4/6)
Survival time of control animals (d, 9.0±0.0)				
L1210 leukemia	6.25	103	98	98
i.p.–*p.o.*	12.5	98	100	103
q4d d 1—9	25.0	98	100	105
	50.0	98	114	119
	100.0	98	132	151
	200.0	98	160	199
	400.0	103	192	240
Survival time of control animals (d, 7.3±0.0)				
B16 melanoma	3.12	104	107	99
i.p.–i.p.	6.25	104	101	109
qd d 1—17	12.5	109	107	111
	25.0	107	106	117
	50.0	121	103	138
	100.0	139	113	155
	200.0	153	144	158
	400.0	179	171	215 (2/6)
Survival time of control animals (d, 22.5±1.5)				
B16 melanoma	12.5	107	111	109
i.p.–i.v.	25.0	112	111	112
q4d d 1—17	50.0	115	117	121
	100.0	121	118	123
	200.0	121	118	132
	400.0	125	117	143
Survival time of control animals (d, 22.5±1.5)				

a) T/C% = (mean survival time of the tested animals)/(mean survival time of the control animals) × 100. *b*) Number of cured mice/number of mice treated.

Table III. Antitumor Activity of the Selected Derivatives against Solid Tumors

Tumor system	Total dose (mg/kg)	I.R.%[a]		
		6-01	6-09	6-27
Sarcoma 180	50.0	27.8	38.5	59.0[b,c]
s.c.–i.v.	100.0	40.0	59.0	74.4[d]
q4d d 1—9	200.0	72.2[d]	69.2[c]	92.3[d]
	400.0	94.8[d]	89.7[d]	—[e]
			(3/10)[f]	
Tumor weight of control animals (g, 1.15±0.7)				
Sarcoma 180	50.0	7.9	11.4	14.3
s.c.–*p.o.*	100.0	0	72.9[d]	59.8[c]
q4d d 1—9	200.0	29.3	69.3[d]	80.0[d]
	400.0	26.4	77.9[d]	90.7[d]
Tumor weight of control animals (g, 1.40±0.82)				
Meth A	50.0	3.8	45.1	51.0[d]
s.c.–i.v.	100.0	31.3	49.1[d]	74.0[d]
q4d d 1—9	200.0	51.8[d]	53.9[d]	90.3[d]
	400.0	86.3[d]	67.5[d]	98.5[d]
Tumor weight of control animals (g, 2.06±0.8)				
Meth A	50.0	24.2	38.2[c]	37.6[c]
s.c.–i.v.	100.0	27.9	33.9[c]	46.1[d]
q4d d 1—9	200.0	26.1	52.7[d]	73.3[d]
	400.0	36.4	60.6[d]	90.3[d]
	600.0	—	—	94.5[d]
	800.0	—	—	97.3[d]
	1000.0	—	—	99.2[d]
				(6/10)
Tumor weight of control animals (g, 1.65±0.4)				

a) Inhibition rate (I.R.)% =

$$\left(1-\frac{\text{mean tumor weight of tested animals}}{\text{mean tumor weight of control animals}}\right)\times 100.$$

b) Statistical significance of difference from control, *c*) $p<0.01$, *d*) $p<0.001$ (Student's *t* test). *e*) Not tested. *f*) Number of tumor-free mice/number of mice tested.

We selected three water-soluble derivatives, **6-01**, **09**, and **27** for further evaluation of their activity of several tumor systems (Tables II and III). The compounds showed good activity in these tumor systems, but compounds **6-01** and **09** were inactive by oral administrations (*p.o.*) and on B16 melanoma by intravenous (i.v.) administration. Compound **6-27** had satisfactory activity in all systems examined.

The highlighting of the carbamate linkage is susceptible to hydrolysis mainly in serum and liver, in this case, that is, CPT-11 was gradually converted into compound **4**. On the administration of CPT-11 in mice, a rather higher concentration of **4** is sustained for a longer term than by the administration of the sodium salt of **4** in serum and some tissues.[14] The compound derived the excellent activity because *Camptotheca* alkaloids were said to exert the activity time-dependently.[7b]

Among the 36 derivatives, there were great differences in their activity depending on the structure of the side chains which held the diamine-monocarbamate moiety. The diamine side chain furnishes the derivatives with a more lipophilic property and also endows a specific structure as the substrate for the hydrolyzing enzyme. Characterization of the enzyme is now being undergone. We intend to measure the hydrolysis profile of the enzyme on derivatives synthesized herein to understand the structure–activity relationships.

In conclusion, we synthesized here the E ring intact water-soluble derivatives of compound **4** by connecting with diamines through carbamate linkage and evaluated the compound in mice tumor screens. CPT-11, hydrochloride trihydrate of **6-27**, was found as a potential nominee for a new anticancer agent.

A synthesis of other types of water-soluble derivatives of compound **4** on this line is now under progress. Synthesis and activity of sulfates, phosphates and glycosides of **4** will be published elsewhere.

Experimental

Melting points (with decomposition) were uncorrected. Ultraviolet (UV) spectra were taken with a Shimadzu UV-240 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 (400 MHz) spectrometer with tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on a Shimadzu IR 435 and FTIR-5000 (Japan Spectroscopic Co., Ltd.). Mass spectra (MS) were measured with a Hitachi M-80B Mass Spectra. Optical rotation was taken with a DIP-360 Digital Polarimeter (Japan Spectroscopic Co., Ltd.) with a Thermostat Model RCS 6-D (Messengeraete-Werk Lauda, West Germany). X-Ray crystallography was conducted with a Rigaku AFC-5 Diffractometer.

7-Ethylcamptothecin (2) To a suspension of **1** (1.00 g, 2.9 mmol) in water (100 ml) containing $FeSO_4 \cdot 7H_2O$ (300 mg, 1.1 mmol) and propionaldehyde (2 ml), conc. H_2SO_4 (11 ml) was dropwise added in an ice bath. To the mixture 30% H_2O_2 (720 mg, 6.4 mmol) was added with stirring. The stirring was continued for 3 h at room temperature. The mixture was diluted with H_2O and the suspension was extracted with $CHCl_3$ (100 ml × 3). The extracts were evaporated and the residue was

passed through silica gel column chromatography with 2% MeOH–$CHCl_3$. **2**: 0.84 g, 77% yield as pale yellow needles, mp 258—261 °C [EtOH]. IR (KBr) ν: 1750, 1650, 1595 cm^{-1}. ^{1}H-NMR ($CDCl_3$–DMSO-d_6) δ: 0.97 (3H, t, J=7 Hz), 1.39 (3H, t, J=7 Hz), 1.91 (2H, q, J=7 Hz), 3.21 (2H, q, J=7 Hz), 521 (2H, s), 5.24, 5.57 (two 1H's, d, J=16 Hz), 7.49 (1H, s), 7.44—8.21 (4H, m). MS m/z: 376.1399 [M^+]. *Anal.* Calcd for $C_{22}H_{20}N_2O_4$: C, 70.20; H, 5.36; N, 7.44. Found: C, 70.37; H, 5.35; N, 7.39.

7-Ethylcamptothecin 1-Oxide (3) A solution of **2** (3.00 g, 8.0 mmol) and 30% H_2O_2 (50 ml) in acetic acid (800 ml) was heated at 70—80 °C for 3.5 h. The mixture was condensed to about one third at 45—55 °C and the residue was poured into ice-water (3 l). The precipitated material in the solution was collected by suction. The filtered material was purified by recrystallization. **3**: 2.4 g, 78% yield as yellow-orange needles, mp 255 °C [$CHCl_3$–n-C_6H_{14}]. ^{1}H-NMR (DMSO-d_6) δ: 0.87 (3H, t, J=7 Hz), 1.28 (3H, t, J=7 Hz), 1.84 (2H, q, J=7 Hz), 3.10 (2H, q, J=7 Hz), 5.26 (2H, s), 5.36 (2H, s), 6.24 (1H, s), 7.80 (3H, m), 8.19 (1H, s), 8.35 (1H, m). MS m/z: 392 [M^+] for $C_{22}H_{20}N_2O_5$=392.

7-Ethyl-10-hydroxycamptothecin (4) A solution of **3** (1.00 g, 2.6 mmol) and 1 N H_2SO_4 (2.6 ml) in dioxane (1 l) was degassed by bubbling N_2 for 20 min. The mixture was irradiated by a high pressure Hg lamp (450 W, Usio UM-452) with a Pyrex filter for 30 min under stirring. The reaction mixture was evaporated to dryness and the residue was dissolved in 10% MeOH–$CHCl_3$ (50 ml). The solution was washed with water (500 ml) and the insoluble material of both phases was collected on a Celite pad by suction and the Celite pad was eluted with 10% MeOH–$CHCl_3$ (200 ml × 3). The eluents were condensed and the residue was washed with MeOH and then recrystallized. **4**: 0.5 g, 49% yield as pale yellow needles, mp 216 °C [EtOH]. IR (KBr) ν: 3600, 3400, 3220, 2970, 1760, 1650, 1590, 1570, 1510 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, J=7 Hz), 1.38 (3H, t, J=7 Hz), 1.90 (2H, q, J=7 Hz), 3.08 (2H, q, J=7 Hz), 5.17 (2H, s), 5.23, 5.54 (two 1H's d, J=16 Hz), 7.34—7.39 (3H, m), 7.92 (1H, d, J=9 Hz). MS m/z: 392 [M^+]. *Anal.* Calcd for $C_{22}H_{20}N_2O_5 \cdot H_2O$: C; 64.38, H; 5.40, N; 6.83. Found: C; 64.24, H; 5.31, N; 6.68.

General Procedure for the Preparation of Carbamate Linked Derivatives (6) by the Reaction of 4 with Chlorocarbonyldiamines To a benzene solution of diamine (60 g), phosgen dimer (50 ml) in benzene was dropwise added with stirring under N_2 atmosphere below 10 °C. The stirring was continued for an additional hour at an ambient temperature. The precipitates were collected on a filter paper by suction. The collected material was dissolved in CH_2Cl_2 and the solution was washed with 7% $NaHCO_3$, dried over $MgSO_4$, filtered, and then evaporated to dryness *in vacuo*. The residue was passed through a silica gel column with CH_2Cl_2–acetone (500 : 1).

The solution of compound **4** and chlorocarbonyl diamine (1.1 eq) in pyridine was stirred for 15 h at an ambient temperature. The mixture was evaporated to dryness *in vacuo*. The residue was dissolved in CH_2Cl_2 and the solution was shaken with 7% $NaHCO_3$, the organic layer was dried over $MgSO_4$, filtered, and condensed *in vacuo*. The residue was purified through a silica gel column with MeOH–$CHCl_3$ (1 : 20).

Compounds **6-09**, **10**, **11**, **23**—**27**, and **28** were obtained by this method.

General Procedure for the Preparation of 6 by the Reaction of 7-Ethyl-10-chlorocarbonyloxycamptothecin (5) with Diamines: **4** (5.00 g, 12.8 mmol) was dissolved in dioxane (10 l) containing triethylamine (50 ml), and phosgen [generated from phosgen dimer (3.75 ml) on active charcoal] was passed through the mixture with stirring at room temperature. The stirring was continued for an additional hour. The solution was filtered by suction and the filtrate was condensed to dryness *in vacuo*. The residue was triturated with acetone and collected on a filter paper to afford **5** (5.2 g, 90.0% yield) as colorless powder.

5 (3.0 g) was dissolved in a mixture of CH_2Cl_2–MeOH (500 ml–150 ml) containing pyridine (15 ml), and then diamine (2.0 eq) in CH_2Cl_2 was added dropwise to the mixture with stirring. The mixture was stirred for 15 h at room temperature and was condensed to dryness *in vacuo*. The residue was dissolved in CH_2Cl_2 and the solution was washed with 7% $NaHCO_3$ and then the organic layer was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was purified through silica gel column chromatography with 4% MeOH–CH_2Cl_2.

Carbamate linked derivatives (**6**) other than described above were obtained by this method.

6-01: 25.5% yield as pale yellow needles, mp 228—230 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3430, 2960, 1745, 1718, 1660, 1590, 1413 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.90 (3H, t, J=7 Hz), 1.32 (3H, t, J=7 Hz), 1.97 (2H, q, J=7 Hz), 3.04—3.65 (10H, m), 5.32 (2H, s), 5.44 (2H, s), 6.50 (1H, s), 7.34 (1H, s), 7.66 (1H, dd, J=2, 8 Hz), 7.97 (1H, d, J=2 Hz), 8.16 (1H, d, J=8 Hz). *Anal.* Calcd for $C_{27}H_{28}N_4O_6 \cdot H_2O$: C, 62.05; H, 5.79; N, 10.72. Found: C, 62.05; H, 5.42; N, 10.96.

6-02: 54.2% yield as pale yellow needles, mp 236—239 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3430, 2970, 1743, 1715, 1655, 1598, 1459 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, t, J=7 Hz), 1.29 (3H, t, J=7 Hz), 1.87 (2H, q, J=7 Hz), 2.25 (3H, s), 3.18 (2H, q, J=7 Hz), 3,49—3.64 (8H, m), 5.31 (2H, s), 5.43 (2H, s), 6.50 (1H, s), 7.31 (1H, s), 7.64 (1H, dd, J=2, 9 Hz), 7.97 (1H, d, J=2 Hz), 8.15 (1H, d, J=9 Hz). MS m/z 518 [M^+]. *Anal.* Calcd for $C_{30}H_{34}N_4O_7 \cdot 1/2H_2O$: C, 63.75; H, 5.92; N, 10.62. Found: C, 63.87; H, 5.74; N, 10.71.

6-03: 75.3% yield as pale yellow needles, mp 200—203 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3430, 2960, 1742, 1720, 1655, 1597, 1412 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.90 (3H, t, J=7 Hz), 1.06 (3H, t, J=7 Hz), 1.32 (3H, t, J=7 Hz), 1.90 (2H, q, J=7 Hz), 2.42 (2H, q, J=7 Hz), 2.18—3.17 (10 H, m), 5.32 (2H, s), 5.44 (2H, s), 6.48 (1H, s), 7.35 (1H, s), 7.66 (1H, dd, J=2, 8 Hz), 7.99 (1H, d, J=2 Hz), 8.18 (1H, d, J=8 Hz). MS m/z: 532 [M^+] for $C_{29}H_{32}N_4O_6$=532.

6-04: 34.7% yield as pale yellow needles, mp 210—213 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3440, 2960, 1750, 1720, 1655, 1598, 1412 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, t, J=7 Hz), 1.02 (3H, t, J=7 Hz), 1.38 (3H, t, J=7 Hz), 1.50—1.60 (2H, m), 1.87 (2H, q, J=7 Hz), 2.51 (2H, q, J=7 Hz), 3.11 (2H, q, J=7 Hz), 3.50—3.90 (8H, m), 5.16 (2H, s), 5.23 (1H, d, J=16 Hz), 5.47 (1H, d, J=16 Hz), 7.45 (1H, dd, J=2, 8 Hz), 7.50 (1H, s), 7.70 (1H, d, J=2 Hz), 8.07 (1H, d, J=8 Hz). MS m/z: 546 [M^+] for $C_{30}H_{34}N_4O_6$=546.

6-05: 29% yield as pale yellow needles, mp 226—230.5 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr)ν: 3400, 2950, 1750, 1715, 1650, 1600, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.40—2.20 (9H, br), 1.80—2.20 (2H, m), 2.50—2.70 (4H, br m), 3.16 (2H, q, J=7 Hz), 3.60—3.90 (4H, br m), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.60 (1H, dd, J=2, 8 Hz), 7.64 (1H, s), 7.85 (1H, d, J=2 Hz), 8.22 (1H, d, J=8 Hz).

6-06: 81.8% yield as pale yellow needles, mp 160—162 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3440, 2940, 1745, 1720, 1655, 1600, 1415 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.89 (3H, t, J=7 Hz), 1.29 (3H, t, J=7 H), 1.87 (2H, q, J=7 Hz), 3.19 (2H, q, J=7 Hz), 3.56 (2H, s), 3.50—3.70 (8H, m), 5.32 (2H, s), 5.43 (2H, s), 6.50 (1H, s), 7.32 (1H, s), 7.34 (5H, br s), 7.45 (1H, dd, J=2, 8 Hz), 7.97 (1H, d, J=2 Hz), 8.16 (1H, d, J=8 Hz). *Anal.* Calcd for $C_{34}H_{34}N_4O_6 \cdot 1/2H_2O$: C, 66.65; H, 5.92; N,9.14.

6-07: 48.5% yield as pale yellow powder, mp 228—230 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3300, 2940, 1745, 1709, 1655, 1592, 1412 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.89 (3H, t, J=7 Hz), 1.30 (3H, t, J=7 Hz), 1.63 (2H, m), 1.88 (2H, q, J=7 Hz), 3.20—3.65 (14H, m), 5.32 (2H, s), 5.43 (2H, s), 6.51 (1H, s), 7.32 (1H, s), 7.65 (1H, dd, J=2, 8 Hz), 7.98 (1H, d, J=2 Hz), 8.17 (1H, d, J=8 Hz).

6-08: 23% yield as pale yellow powder, mp 198—200 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3390, 2960, 1745, 1715, 1655, 1595, 1455 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 2.64 (4H, t, J=4 Hz), 2.68 (2H, t, J=4 Hz), 3.16 (2H, q, J=7 Hz), 3.63 (2H, t, J=3 Hz), 3.70 (2H, t, J=5.5 Hz), 3.73 (6H, m), 5.25 (2H, s), 5.29 (1H, d, J=16 Hz), 5.74 (2H, d, J=16 Hz), 7.58 (1H, dd, J=2, 8 Hz), 7.62 (1H, s), 7.85 (1H, d, J=2 Hz), 8.21 (1H, d, J=8 Hz).

6-09: 75.9% yield as pale yellow needles, mp 237—240 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3420, 3340, 2960, 1750, 1720, 1655, 1595 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.89 (3H, t, J=7 Hz), 1.09 (6H, d, J=6 Hz), 1.30 (3H, t, J=7 Hz), 1.88 (2H, q, J=7 Hz), 2.60 (4H, br s), 3.40—3.70 (4H, m), 3.70—4.00 (1H, m), 5.32 (2H, s), 5.43 (2H, s), 6.50 (1H, s), 7.32 (1H, s), 7.65 (1H, dd, J=2, 8 Hz), 7.98 (1H, d, J=2 Hz), 8.16 (1H, d, J=8 Hz). *Anal.* Calcd for $C_{32}H_{37}N_5O_7 \cdot H_2O$: C, 61.88; H, 6.33; N, 11.28. Found: C, 61.89; H, 6.33; N, 11.28.

6-10: 78.5% yield as pale yellow needles, mp 165.5—166.5 °C [EtOH]. IR (KBr) ν: 3395, 2955, 1745, 1715, 1655, 1615, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, t, J=7 Hz), 1.40 (3H, t, J=7 Hz), 1.80—1.95 (2H, m), 1.95—2.05 (4H, m), 2.65—2.80 (4H, m), 3.16 (2H, q, J=7 Hz), 3.23 (2H, s), 3.50 (4H, dd, J=7, 14 Hz), 3.65—3.85 (4H, m), 5.25 (2H, s), 5.30 (1H, d, J=16 Hz), 5.74 (1H, d, J=16 Hz), 7.58 (1H, dd, J=2, 9 Hz), 7.65 (1H, s), 7.84 (1H, d, J=2 Hz), 8.21 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{33}H_{37}N_5O_8 \cdot H_2O$: C, 62.55; H, 6.20; N, 11.05. Found: C, 62.45; H, 6.05; N, 11.12.

6-11: 62.7% yield and pale yellow needles, mp 205.5—208 °C [EtOH]. IR (KBr) ν: 3400, 2960, 1745, 1710, 1650, 1595, 1455 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.40 (3H, t, J=7 Hz), 1.61 (2H, m), 1.80—2.00 (2H, m), 1.90—2.05 (2H, m), 2.46 (1H, m), 2.61 (4H, m), 2.96 (1H, br m), 3.13 (1H, m), 3.16 (2H, q, J=7 Hz), 3.76 (4H, t, J=3.5 Hz), 3.85 (1H, br s), 4.23 (1H, d, J=13 Hz), 4.42 (1H, d, J=13 Hz), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.58 (1H, dd, J=2,

9 Hz), 7.65 (1H, s), 7.83 (1H, d, J=2 Hz), 8.22 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{33}H_{37}N_5O_8 \cdot 1/2H_2O$: C, 61.86; H, 5.98; N, 10.93. Found: C, 62.06; H, 5.78; N, 10.94.

6-12: 38.0% yield as pale yellow needles, mp 202—204 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2960, 1745, 1715, 1655, 1600, 1510, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.42 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 3.10—3.30 (4H, m), 3.17 (2H, q, J=7 Hz), 3.80—4.00 (4H, m), 5.27 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.04—7.45 (5H, br s), 7.62 (1H, dd, J=2, 8 Hz), 7.65 (1H, s), 7.91 (1H, d, J=2 Hz), 8.21 (1H, d, J=8 Hz). MS m/z: 580 [M$^+$] for $C_{33}H_{32}N_4O_6$=580.

6-13: 36% yield as pale yellow powder, mp 241—242 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2960, 1743, 1715, 1655, 1600, 1510, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.42 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 3.10—3.30 (4H, m), 3.16 (2H, q, J=7 Hz), 3.80—4.00 (4H, m), 5.27 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.04—7.42 (4H, m), 7.62 (1H, dd, J=2, 8 Hz), 7.65 (1H, s), 7.88 (1H, d, J=2 Hz), 8.24 (1H, d, J=8 Hz).

6-14: 33% yield as pale yellow powder, mp 159—163 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2960, 1750, 1720, 1655, 1590, 1455 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 3.16 (2H, q, J=7 Hz), 3.30 (4H, m), 3.70—3.90 (4H, m), 5.24 (2H, s), 5.29 (1H, d, J=16 Hz), 5.72 (1H, d, J=16 Hz), 6.83—7.20 (4H, m), 7.59 (1H, dd, J=2, 8 Hz), 7.62 (1H, s), 7.87 (1H, d, J=2 Hz), 8.22 (1H, d, J=8 Hz).

6-15: 31% yield as pale yellow powder, mp 179—180.5 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3410, 2970, 1740, 1715, 1660, 1595, 1490 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 3.17 (2H, q, J=7 Hz), 3.20—3.30 (4H, m), 3.70—4.00 (4H, m), 5.26 (1H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 6.90 (2H, d, J=9 Hz), 7.26 (2H, d, J=9 Hz), 7.61 (1H, dd, J=2, 8 Hz), 7.87 (1H, d, J=2 Hz), 8.24 (1H, d, J=8 Hz).

6-16: 63.3% yield as pale yellow needles, mp 156—158 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3440, 2970, 1745, 1720, 1658, 1600, 1515, 1415 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.89 (3H, t, J=7 Hz), 1.30 (3H, t, J=7 Hz), 1.88 (2H, q, J=7 Hz), 3.14 (6H, br s), 5.26 (1H, s), 5.44 (2H, s), 6.50 (1H, s), 6.91 (4H, m), 7.32 (1H, s), 7.69 (1H, dd, J=2, 8 Hz), 8.01 (1H, d, J=2 Hz), 8.18 (1H, d, J=8 Hz).

6-17: 38% yield as pale yellow needles, mp 230.5—232.5 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2960, 1745, 1720, 1655, 1585, 1540, 1490 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 3.17 (2H, q, J=7 Hz), 5.26 (1H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 6.58 (1H, t, J=5 Hz), 7.62 (1H, dd, J=2, 8 Hz), 7.65 (1H, s), 7.87 (1H, d, J=2 Hz), 8.23 (1H, d, J=8 Hz), 8.37 (2H, d, J=5 Hz). MS m/z: 581 [M$^+$] for $C_{32}H_{31}N_5O_6$=581.

6-18: 34% yield as pale yellow needles, mp 229—230.5 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2960, 1745, 1720, 1655, 1590, 1475 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 3.17 (2H, q, J=7 Hz), 3.60—4.00 (8H, m), 5.26 (1H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 6.70 —6.72 (2H, m), 7.55—7.61 (2H, m), 7.61 (1H, dd, J=2, 8 Hz), 7.65 (1H, s), 7.87 (1H, d, J=2 Hz), 8.24 (1H, d, J=8 Hz).

6-19: 36% yield as pale yellow powder, mp 220.5—221.5 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2960, 1740, 1710, 1660, 1590, 1510 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.14 (two 1.5H, s), 1.41 (3H, t, J=7 Hz), 1.80—2.20 (2H, br m), 2.69—2.93 (2H, br m), 3.09—4.16 (4H, br m), 3.16 (2H, q, J=7 Hz), 5.24 (2H, s), 5.29 (1H, d, J=16 Hz), 5.73 (1H, d, J=16 Hz), 7.58 (1H, dd, J=2, 8 Hz), 7.62 (1H, s), 7.85 (1H, d, J=2 Hz), 8.21 (1H, d, J=8 Hz). MS m/z: 518 [M$^+$] for $C_{28}H_{30}N_4O_6$=518.

6-20: 32% yield as pale yellow powder, mp 215.5—217 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2960, 1740, 1710, 1660, 1590, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$)δ: 1.03 (3H, t, J=7 Hz), 1.32 (3H, d, J=7 Hz), 1.41 (3H, t, J=4 Hz), 1.42 (3H, d, J=7 Hz), 1.80—2.20 (2H, br m), 2.64 (1H, m), 3.17 (2H, q, J=7 Hz), 3.27 (1H, m), 3.37 (1H, dd, J=2, 14 Hz), 3.52 (1H, dd, J=2, 14 Hz), 3.79 (1H, m), 4.35 (1H, m), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.74 (1H, d, J=16 Hz), 7.58 (1H, dd, J=2, 8 Hz), 7.66 (1H, s), 7.84 (1H, d, J=2 Hz), 8.23 (1H, d, J=8 Hz). MS m/z: 532 [M$^+$] for $C_{29}H_{32}N_4O_6$=532.

6-21: 23% yield as pale yellow powder, mp 216—227 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2980, 1750, 1720, 1660, 1610, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.14 (3H, d, J=7 Hz), 1.16 (3H, d, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1,80—2.00 (2H, br m), 2.52 (1H, t, J=12 Hz), 2.69 (1H, t, J=12 Hz), 3.16 (2H, q, J=7 Hz), 4.15 (2H, d, J=12 Hz), 4.23 (2H, d, J=12 Hz), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.59 (1H, dd, J=2, 8 Hz), 7.64 (1H, s), 7.85 (1H, d, J=2 Hz), 8.22 (1H, d, J=8 Hz).

6-22: 36% yield as pale yellow needles, mp 235—237 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3370, 2955, 1745, 1700, 1655, 1595, 1410 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.50—2.00 (4H, m), 1.80—2.00 (2H, m), 2.34 (6H, s), 2.40 (1H, m), 2.34 (1H, br t, J=12 Hz), 3.11 (1H, br t, J=12 Hz), 3.16 (2H, q, J=7 Hz), 3.82 (1H, br s), 4.32 (1H, br), 4.41 (1H, br), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.59 (1H, dd, J=2, 9 Hz), 7.64 (1H, s), 7.84 (1H, d, J=2 Hz), 8.22 (1H, d, J=9 Hz).

6-23: 45% yield as pale yellow needles, mp 236—238 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3385, 2955, 1745, 1710, 1655, 1595, 1410 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.11 (6H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.50—2.00 (4H, m), 1.80—2.00 (2H, m), 2.66 (4H, q, J=7 Hz), 2.82 (1H, m), 2.91 (1H, br t, J=12 Hz), 3.07 (1H, br t, J=12 Hz), 3.16 (2H, q, J=7 Hz), 3.80 (1H, br s), 4.36 (1H, br), 4.45 (1H, br), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.59 (1H, dd, J=2, 9 Hz), 7.64 (1H, s), 7.84 (1H, d, J=2 Hz), 8.22 (1H, d, J=9 Hz).

6-24: 48% yield as pale yellow needles, mp 196—199 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3395, 2945, 1745, 1715, 1655, 1600, 1455 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (6H, t, J=7 Hz), 1.04 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.46 (4H, sex, J=7 Hz), 1.50—2.00 (4H, m), 1.80—2.00 (2H, m), 2.45 (4H, t, J=7 Hz), 2.73 (1H, m), 2.88 (1H, br t, J=12 Hz), 3.04 (1H, br t J=12 Hz), 3.16 (2H, q, J=7 Hz), 3.86 (1H, br s), 4.35 (1H, br), 4.45 (1H, br), 5.25 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.59 (1H, dd, J=2, 9 Hz), 7.65 (1H, s), 7.84 (1H, d, J=2 Hz), 8.22 (1H, d, J=9 Hz).

6-25: 52% yield as pale yellow needles, mp 203.5—206.5 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3390, 2940, 1750, 1715, 1665, 1610, 1455 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.93 (6H, t, J=7 Hz), 1.04 (3H, t, J=7 Hz), 1.33 (4H, sex, J=7 Hz), 1.35—1.50 (4H, m), 1.41 (3H, t, J=7 Hz), 1.16 (4H, sex, J=7 Hz), 1.50—2.00 (4H, m), 1.80—2.00 (2H, m), 2.48 (4H, t, J=7 Hz), 2.71 (1H, m), 2.88 (1H, br t, J=13 Hz), 3.04 (1H, br t, J=13 Hz), 3.16 (2H, q, J=7 Hz), 3.85 (1H, br s), 4.35 (1H, br), 4.44 (1H, br), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.59 (1H, dd, J=2, 9 Hz), 7.65 (1H, s), 7.84 (1H, d, J=2 Hz), 8.22 (1H, d, J=9 Hz).

6-26: 58.2% yield as pale yellow powder, mp 219—223 °C [n-$C_{16}H_{14}$–$CHCl_3$]. IR (KBr) ν: 3400, 2955, 1745, 1715, 1655, 1600, 1450 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7 Hz), 1.40 (3H, t, J=7 Hz), 1.66 (2H, br t, J=7 Hz), 1.84 (4H, br s), 1.80—2.00 (2H, m), 1.95—2.20 (2H, br s), 2.30 (1H, br), 2.64 (4H, br s), 3.04 (1H, br), 3.15 (2H, q, J=7 Hz), 3.18 (1H, br), 3.95 (1H, br s), 4.22 (1H, br d, J=14 Hz), 4.33 (1H, br d, J=14 Hz), 5.25 (2H, s), 5.30 (1H, d, J=16 Hz), 5.74 (1H, d, J=16 Hz), 7.57 (1H, dd, J=2, 9 Hz), 7.64 (1H, s), 7.82 (1H, d, J=2 Hz), 8.22 (1H, d, J=9 Hz).

6-27: 79.8% yield as pale yellow powder, mp 222—223 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3420, 2930, 1745, 1715, 1655, 1600, 1412 cm^{-1} ^{1}H-NMR (DMSO-d_6) δ: 0.90 (3H, t, J=7 Hz), 1.28 (3H, t, J=8 Hz), 1.38 (2H, br), 1.49 (6H, br), 1.78 (2H, br d), 1.85 (1H, q, J=7 Hz), 1.90 (1H, q, J=7 Hz), 2.46 (5H, br), 2.89 (1H, br t), 3.05 (1H, br t), 3.14 (2H, q, J=8 Hz), 4.31 (1H, br d), 4.24 (1H, br d), 5.24 (2H, s), 5.42 (2H, s), 6.51 (1H, s), 7.29 (1H, s), 7.60 (1H, dd, J=3, 9 Hz), 7.90 (1H, d, J=3 Hz), 8.09 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{33}H_{38}N_4O_6$: C, 61.88; H, 6.39; N, 9.38. Found: C, 65.28; H, 6.39; N, 9.38.

6-28: 52.4% yield as pale yellow powder, mp 221.5—223 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2950, 1745, 1710, 1655, 1595, 1410 cm^{-1} ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.40 (3H, t, J=7 Hz), 1.61 (2H, br t, J=4 Hz), 1.80—2.00 (2H, m), 1.90—2.05 (2H, m), 2.46 (1H, m), 2.61 (4H, t, J=4 Hz), 2.96 (1H, br), 3.13 (1H, br), 3.16 (2H, q, J=7 Hz), 3.76 (4H, t, J=4 Hz), 3.85 (1H, br s), 4.32 (1H, d, J=12 Hz), 4.42 (1H, d, J=4 Hz), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.58 (1H, dd, J=2, 9 Hz), 7.65 (1H, s), 7.83 (1H, d, J=2 Hz), 8.22 (1H, d, J=9 Hz).

6-29: 60.8% yield as pale yellow powder, mp 159—162 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3420, 2940, 1745, 1720, 1656, 1600, 1405 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.08—2.15 (6H, m), 2.04 (3H, s), 3.00—3.20 (6H, m), 4.12 (2H, q, J=7 Hz), 5.25 (2H, s), 5.32 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.59 (1H, dd, J=2, 8 Hz), 7.65 (1H, s), 7.85 (1H, d, J=2 Hz), 8.22 (1H, d, J=8 Hz).

6-30: 31% yield as pale yellow powder, mp 222—223.5 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2970, 1750, 1700, 1660, 1590, 1460 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, t, J=7 Hz), 1.43 (3H, t, J=7 Hz), 1.80—2.00 (4H, m), 2.98—3.00 (2H, m), 3.04—3.10 (2H, m), 3.16 (2H, q, J=7 Hz), 3.64 (2H, t, J=4.5 Hz), 3.69 (2H, t, J=4.5 Hz), 5.24 (2H, s), 5.29 (1H, d, J=16 Hz), 5.74 (1H, d, J=16 Hz), 7.59 (1H, dd, J=2, 8 Hz), 7.62 (1H, s), 7.78 (1H, d, J=2 Hz), 8.21 (1H, d, J=8 Hz).

6-31: 39% yield as pale yellow powder, mp 220—222.5 °C [n-C_6H_{14}–

$CHCl_3$]. IR (KBr) ν: 3400, 1750, 1710, 1660, 1590, 1460 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.80—2.00 (4H, m), 2.42, 2.44 (two 1.5H, s), 2.66—2.77 (4H, m), 3.14 (2H, q, J=7 Hz), 3.68—3.81 (4H, m), 5.24 (2H, s), 5.29 (1H, d, J=16 Hz), 5.73 (1H, d, J=16 Hz), 7.59 (1H, dd, J=2, 8 Hz), 7.62 (1H, s), 7.86 (1H, d, J=2 Hz), 8.21 (1H, d, J=8 Hz). MS m/z: 532 [M^+] for $C_{29}H_{32}N_4O_6$=532.

6-32: 48.9% yield as pale yellow powder, mp 202—203.1 °C [n-C_6H_{14}–$CHCl_3$]. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, t, J=7 Hz), 1.39 (3H, t, J=7 Hz), 1.84 (2H, q, J=7 Hz), 2.36 (6H, br s), 2.64 (2H, t, J=7 Hz), 3.09 (1.5H, s), 3.22 (1.5H, s), 3.16 (2H, q, J=7 Hz), 3.58 (2H, t, J=7 Hz), 5.24 (2H, s), 5.27 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.26 (1H, s), 7.41 (1H, dd, J=2, 9 Hz), 7.62 (1H, d, J=2 Hz), 8.09 (1H, d, J=9 Hz).

6-33: 28% yield as pale yellow needles, mp 172—175 °C [n-C_6H_{14}–$CHCl_3$]. IR(KBr)ν: 3400, 2970, 1745, 1715, 1660, 1610, 1460 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.07 (6H, t, J=7 Hz), 1.40 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 2.60 (4H, q, J=7 Hz), 2.74 (2H, m), 2.74 (2H, m), 3.09 (1.5H, br s), 3.21 (1.5H, br s), 3.15 (2H, q, J=7 Hz), 5.29 (1H, d, J=16 Hz), 5.73 (1H, d, J=16 Hz), 7.59 (1H, dd, J=2, 8 Hz), 7.62 (1H, s), 7.85 (1H, d, J=2 Hz), 8.20 (1H, d, J=8 Hz). MS m/z: 548 [M^+] for $C_{30}H_{36}N_4O_6$=548.

6-34: 17% yield as pale yellow powder, mp 166—169 °C [n-C_6H_{14}–$CHCl_3$]. IR (KBr) ν: 3400, 2965, 1750, 1715, 1650, 1610, 1460 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7 Hz), 1.07 (3H, t, J=7 Hz), 1.08 (3H, t, J=7 Hz), 1.26 (3H, t, J=7 Hz), 1.34 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 2.61 (4H, m), 2.70—2.80 (2H, m), 3.16 (2H, q, J=7 Hz), 3.40—3.60 (2H, m), 5.26 (2H, s), 5.31 (1H, d, J=16 Hz), 5.75 (1H, d, J=16 Hz), 7.60 (1H, dd, J=2, 8 Hz), 7.64 (1H, s), 7.83 (1H, d, J=2 Hz), 8.22 (1H, d, J=8 Hz).

6-35: 6% yield as pale yellow syrup. IR ($CHCl_3$) ν:3400, 2955, 1800, 1730, 1650, 1550, 1510, 1460 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, J=7 Hz), 1.00—1.10 (6H, m), 1.04 (3H, t, J=7 Hz), 1.42 (3H, t, J=7 Hz), 1.80—2.00 (2H, m), 3.15 (2H, q, J=7 Hz), 3.40—3.50 (4H, m), 3.50—3.70 (4H, m), 3.87 (2H, t, J=7 Hz), 4.10 (2H, t, J=7 Hz), 5.24 (2H, s), 5.29 (1H, d, J=16 Hz), 5.73 (1H, d, J=16 Hz), 7.58 (1H, dd, J=2, 8 Hz), 7.62 (1H, s), 7.84 (1H, d, J=2 Hz), 8.20 (1H, d, J=8 Hz).

6-36: 8% yield as pale yellow syrup. IR ($CHCl_3$) ν: 3370, 2950, 1745, 1715, 1655, 1460 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.80—4.40 (23H, m), 0.85—1.15 (5H, m), 1.80—2.00 (2H, m), 5.28 (2H, s), 5.35 (1H, d, J=16 Hz), 5.69 (1H, d, J=16 Hz), 7.69 (1H, dd, J=2, 8 Hz), 8.12 (1H, d, J=2 Hz), 8.13 (1H, s), 8.55 (1H, d, J=8 Hz).

Antitumor Activity of Derivatives 6 on L1210 Leukemia, Test Method (Table I) L1210 leukemia cells (10^5) were implanted intraperitoneally (i.p.) in 7 week old CDF_1 female mice on day 0, and 6 mice were used at the each dose. The samples were administered i.p. on days 1, 5, and 9. The control mice were injected with saline. Cured mice were calculated on day 40.

Antitumor Activity of the Selected Derivatives against Acites Tumors, Test Method (Table II) An aliquot of each suspension (P338, 1.7×10^6 cell/ml, L1210, 3×10^5 cell/ml; B16, 2×10^6 cell/ml) was implanted i.p. in 7 week old female mice (CDF_1 for P388 and L1210; C57BL/6 for B16) on day 0. The sample were administered i.p., *p.o.*, and i.v., and the control mice were injected with saline. Cured mice were observed on day 40 for P388 and L1210, day 60 for B16.

Antitumor Activity of the Selected Derivatives against Solid Tumors, Test Method A 0.1 ml aliquot of each tumor suspension (S180, 1×10^6 cells/ml; Meth A, 1×10^5 cells/ml) was implanted subcutaneously in 7 week old female mice (ICR for S180 and BALB/c for Meth A) on day 0. The samples were administered i.v. or *p.o.* on days indicated in Table III. Inhibition rates and cured mice were calculated on day 21.

CPT-11 (+)-(4*S*)-4,11-Diethyl-4-hydroxy-9-[(4-piperidinopiperidino)-carbonyloxy]-1*H*-pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-3,14-(4*H*,12*H*)-dione hydrochloride trihydrate

a) Preparation: To a suspension of **6-27** (1000 g) in distilled water (10.7 l), 5 N HCl (0.4 l) was added and the mixture was warmed at 70 °C. To the solution activated carbon (200 g) was added and the mixture was stirred for 30 min at 70 °C. After cooling the mixture to an ambient temperature, the mixture was stirred with AcOEt (3.7 l). The mixture was filtered through a Celite pad by suction and the aqueous layer was separated and passed through a membrane filter (0.22 μm) and then the filtrate was condensed to about 9 l *in vacuo*. To the solution, 5 N HCl (75 ml) was added and the solution was stirred for 40 h at an ambient temperature. The precipitate was collected and the material was dried *in vacuo*. The dried material was placed in a 75% relative humidity chamber for 70 h.

b) Structural Determination of CPT-11: Slightly pale yellow needles, mp 256.5 °C [H_2O]. UV (EtOH) λ nm (ε): 221 (53800), 254 (36600), 359 (26200), 372 (25300). $[\alpha]_D^{20}$ +67.7° (c=1, water). IR (KBr) ν: 3376 (O–H), 2630, 2546 (NH^+), 1748 (lactone carbonyl), 1688 (carbamate carbonyl), 1663 (pyridone carbonyl), 1613 (quinoline C=N), 1568 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 90 °C) δ: 0.92 (3H, t, J=7 Hz, C_{26}-CH_2CH_3), 1.33 (3H, t, J=8 Hz, C_7-CH_2CH_3), 1.47 (1H, q-like, $C_{4''}$-H_{ax}), 1.73 (1H, d-like, $C_{4''}$-H_{eq}), 1.85 (2H, overlap, $C_{3''}$-H_{ax} and $C_{5''}$-H_{ax}), 1.89 (2H, overlap, $C_{3'}$-H_{ax} and $C_{5'}$-H_{ax}), 1.94 (2H, q, J=7 Hz, C_{20}-CH_2), 2.01 (2H, q-like, $C_{3''}$-H_{eq} and $C_{5''}$-H_{eq}), 2.28 (2H, d-like, $C_{3'}$-H_{eq} and $C_{5'}$-H_{eq}), 2.95 (2H, q-like, $C_{2''}$-H_{ax} and $C_{6''}$-H_{ax}), 3.08 (2H, t-like, $C_{2'}$-H_{ax} and $C_{6'}$-H_{ax}), 3.17 (2H, q, J=8 Hz, C_7-CH_2), 3.42 (3H, br, $C_{4'}$-H, $C_{2''}$-H_{eq} and $C_{6''}$-H_{eq}), 4.31 (2H, d-like, $C_{2'}$-H_{eq} and $C_{6'}$-H_{eq}), 5.27 (2H, s, C_5-H_2), 5.36 (1H, d, J=17 Hz, C_{17}-H_{ax}), 5.45 (1H, d, J=17 Hz, C_{17}-H_{eq}), 7.34 (1H, s, C_{14}-H), 7.65 (1H, dd, J=3, 9 Hz, C_{11}-H), 7.95 (1H, dd, J=3 Hz, C_9-H), 8.13 (1H, d, J=9 Hz, C_{12}-H), 11.10 (1H, br s, N^+-H). ^{13}C-NMR (DMSO-d_6, 90 °C) δ: 7.6 (q, C_{20}-$CH_2\underline{C}H_3$), 13.5 (q, C_7-$CH_2\underline{C}H_3$), 21.7 (t, $C_{4''}$), 22.2 (t, C_7-$\underline{C}H_2CH_3$), 22.4 (t, $C_{3''}$ and $C_{5''}$), 25.7 (t, $C_{3'}$ and $C_{5'}$), 31.0 (t, C_{20}-$\underline{C}H_2CH_3$), 42.9 (t, $C_{2'}$ and $C_{6'}$), 49.2 (t, $C_{2''}$ and $C_{6''}$), 49.4 (t, C_5), 62.2 (d, $C_{4'}$), 65.5 (t, C_{17}), 72.4 (s, C_{20}), 96.6 (d, C_{14}), 114.6 (d, C_9), 119.0 (s, C_{16}), 125.5 (d, C_{11}), 127.1 (s, C_8), 128.2 (s, C_6), 131.0 (d, C_{12}), 145.2 (s, C_7), 146.0 (s, C_3), 146.5 (s, C_{13}), 150.0 (s, C_{10} and/or C_{15}), 150.1 (s, C_{15} and/or C_{10}), 151.8 (s, C_2), 152.6 (s, carbamate carbonyl), 156.9 (s, pyridone carbonyl), 172.1 (s, lactone carbonyl). MS m/z: 586.2786 for

TABLE IV. Fractional Atomic Coordinates and Isotopic Thermal Parameters of CPT-11

Atom	X	Y	Z	B_{eq}
Cl(1)	0.5106 (3)	0.2653 (1)	0.1492 (7)	5.89 (16)
N(2)	0.8520 (10)	0.3943 (2)	1.6368 (20)	3.16 (38)
N(3)	0.3955 (11)	0.3497 (3)	0.8631 (21)	3.61 (41)
N(4)	0.3296 (10)	0.2694 (3)	0.4931 (21)	3.06 (38)
N(5)	0.8362 (11)	0.4438 (2)	2.0741 (21)	3.26 (40)
O(6)	0.3906 (8)	0.3653 (3)	1.2029 (19)	3.88 (35)
O(7)	0.5485 (9)	0.3680 (3)	1.0176 (18)	4.68 (41)
O(8)	0.8177 (11)	0.4750 (2)	2.3576 (22)	4.88 (39)
O(9)	1.1280 (12)	0.4494 (3)	2.5755 (19)	5.62 (46)
O(10)	1.2702 (14)	0.4176 (4)	2.5581 (23)	8.15 (61)
O(11)	1.1818 (11)	0.3897 (3)	2.2213 (27)	6.01 (50)
O(12)	0.0185 (10)	0.3480 (3)	0.7072 (22)	6.19 (44)
O(13)	−0.0168 (14)	0.3044 (4)	0.0060 (23)	8.84 (61)
O(14)	0.0864 (12)	0.3329 (3)	0.3136 (26)	7.14 (50)
C(15)	0.7699 (14)	0.3896 (3)	1.4874 (26)	2.85 (46)
C(16)	0.6155 (13)	0.3757 (4)	1.1831 (26)	3.34 (52)
C(17)	0.9987 (16)	0.4144 (4)	2.0119 (25)	3.13 (49)
C(18)	0.7248 (14)	0.4300 (3)	1.8046 (26)	3.11 (47)
C(19)	0.6650 (15)	0.4041 (3)	1.4965 (26)	3.15 (48)
C(20)	0.6437 (13)	0.4248 (3)	1.6609 (30)	3.26 (46)
C(21)	0.2739 (14)	0.3074 (4)	0.7722 (28)	3.44 (51)
C(22)	0.4609 (16)	0.3468 (5)	0.6659 (31)	4.58 (59)
C(23)	0.4360 (12)	0.3614 (4)	1.0427 (34)	3.16 (45)
C(24)	0.8965 (12)	0.4230 (3)	1.9507 (23)	2.22 (44)
C(25)	0.5882 (14)	0.3963 (4)	1.3366 (32)	3.64 (53)
C(26)	0.3353 (13)	0.3027 (3)	0.5768 (26)	2.71 (46)
C(27)	0.8019 (13)	0.3675 (3)	1.3221 (29)	3.36 (48)
C(28)	0.7214 (13)	0.3613 (4)	1.1794 (28)	3.27 (48)
C(29)	0.2832 (12)	0.3414 (5)	0.8468 (40)	4.88 (66)
C(30)	0.8248 (12)	0.4139 (4)	1.7788 (27)	3.06 (50)
C(31)	0.4560 (15)	0.3113 (4)	0.6021 (28)	3.57 (53)
C(32)	0.2202 (14)	0.2637 (4)	0.3780 (32)	3.47 (52)
C(33)	0.9850 (13)	0.4478 (3)	2.3102 (25)	2.59 (45)
C(34)	0.2285 (17)	0.2333 (4)	0.2647 (31)	4.15 (57)
C(35)	0.3462 (16)	0.2453 (4)	0.6450 (30)	3.60 (53)
C(36)	1.0438 (12)	0.4287 (4)	2.1931 (24)	2.57 (44)
C(37)	0.8765 (14)	0.4571 (3)	2.2595 (26)	2.72 (48)
C(38)	1.0280 (18)	0.4630 (4)	2.5046 (32)	4.58 (65)
C(39)	0.7252 (13)	0.4503 (3)	1.9949 (29)	3.00 (47)
C(40)	1.1890 (19)	0.4283 (5)	2.4758 (31)	5.10 (69)
C(41)	0.3588 (20)	0.2133 (5)	0.5444 (37)	5.29 (72)
C(42)	1.2396 (24)	0.4355 (8)	2.1002 (53)	7.45 (89)
C(43)	1.1622 (16)	0.4224 (4)	2.2500 (28)	4.05 (56)
C(44)	0.2505 (21)	0.2061 (5)	0.4178 (42)	5.49 (80)
C(45)	0.5337 (21)	0.4417 (6)	1.6702 (39)	6.38 (81)
C(46)	0.5267 (22)	0.4707 (6)	1.5210 (49)	7.16 (95)
C(47)	1.2396 (31)	0.4677 (12)	2.1100 (72)	10.99 (99)

$C_{33}H_{38}N_4O_6$=586.2784. *Anal.* Calcd for $C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$, C; 58.53, H; 6.70, N; 8.27, Cl; 5.23. Found: C, 58.59, H; 6.43, N; 8.30, Cl; 5.34.

c) Determination of Optical Purity: 1) ^{1}H-NMR: **6-27** or (*RS*)-**6-27** (10 mg) was dissolved in $CDCl_3$ (5 ml), and the shift reagent (13.6 mg) was dissolved in $CDCl_3$ (2 ml). To the solution of **6-27** or (*RS*)-**6-27** (0.5 ml), the shift reagent solution (0.1 ml) was added.

2) Liquic Chromatography; CPT-11 or (*RS*)-CPT-11 (10 mg) was dissolved in $CHCl_3$–EtOH (4 : 1, 50 ml) and 20 μl of the solutions were used for HPLC operation with a UV deterctor (wave length: 254 nm) and CHIRALCEL OD column (4.6 mm × 25 cm, Daicel Chem. Ind. Co., Osaka Japan) at 40 °C. A mixture of *n*-C_6H_{14}–EtOH (1 : 1) containing 0.2 (v/v)% of diethylamine was used as an eluent at 1 ml/min. (*RS*)-CPT-11 revealed two peaks at 11.8 min and 16.7 min, whereas, CPT-11 prepared by the method described above showed one peak at 16.7 min.

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